

Citation for published version:

Chen, Y-C, Betts, J, Walhin, J-P & Thompson, D 2018, 'Adipose tissue responses to breaking sitting in men and women with central adiposity', *Medicine and Science in Sports and Exercise*, vol. 50, no. 10, pp. 2049-2057.
<https://doi.org/10.1249/MSS.0000000000001654>

DOI:

[10.1249/MSS.0000000000001654](https://doi.org/10.1249/MSS.0000000000001654)

Publication date:

2018

Document Version

Peer reviewed version

[Link to publication](https://doi.org/10.1249/MSS.0000000000001654)

Copyright © 2018 by the American College of Sports Medicine. The final publication is available at Medicine & Science in Sports & Exercise via [https://doi.org/ 10.1249/MSS.0000000000001654](https://doi.org/10.1249/MSS.0000000000001654)

University of Bath

Alternative formats

If you require this document in an alternative format, please contact:
openaccess@bath.ac.uk

General rights

Copyright and moral rights for the publications made accessible in the public portal are retained by the authors and/or other copyright owners and it is a condition of accessing publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Adipose tissue responses to breaking sitting in men and women with central adiposity

Running title: Adipose tissue responses to breaking sitting

Yung-Chih Chen¹, James A. Betts¹, Jean-Philippe Walhin¹ and Dylan Thompson¹

Department for Health, University of Bath, United Kingdom¹

Corresponding author:

Professor Dylan Thompson

Department for Health

University of Bath

Bath, BA2 7AY

United Kingdom

Telephone: +44 (0) 1225 38 3177

Email: d.thompson@bath.ac.uk

19 **Abstract**

20 **Purpose:** Breaking prolonged sitting reduces postprandial glucose and insulin concentrations and
21 influences skeletal muscle molecular signalling pathways but it is unknown whether breaking sitting
22 also affects adipose tissue. **Methods:** Eleven central overweight participants (7 men and 4 post-
23 menopausal women) aged 50 ± 5 years (means \pm SD) completed two mixed-meal feeding trials
24 (PROLONGED SITTING *versus* BREAKING SITTING) in a randomised, counterbalanced design.
25 The BREAKING SITTING intervention comprised walking for 2 min every 20 min over 5.5 h. Blood
26 samples were taken at regular intervals to examine metabolic biomarkers and adipokine
27 concentrations. Adipose tissue samples were taken at baseline and at 5.5 h to examine changes in
28 mRNA expression and secretion of selected adipokines *ex-vivo*. **Results:** Postprandial glycaemia and
29 insulinaemia were attenuated by approximately 50% and 40% in BREAKING SITTING compared
30 to PROLONGED SITTING (iAUC: 359 ± 117 *versus* 697 ± 218 mmol·330 min·L⁻¹, $p = 0.001$ and
31 202 ± 71 *versus* 346 ± 150 nmol·330 min·L⁻¹, $p = 0.001$, respectively). Despite these pronounced and
32 sustained differences in postprandial glucose and insulin concentrations, adipose tissue mRNA
33 expression for various genes (IL-6, leptin, adiponectin, PDK4, IRS1/2, PI3K and Akt1, etc.) and *ex-*
34 *vivo* adipose tissue secretion of IL-6, leptin and adiponectin were not different between trials.
35 **Conclusions:** This study demonstrates that breaking sitting with short bouts of physical activity has
36 very pronounced effects on systemic postprandial glucose and insulin concentrations but this does
37 not translate into corresponding effects within adipose tissue.

38

39 **Key words:** Sedentary, physical activity, gene expression, postprandial, insulin signalling

40

41

42 INTRODUCTION

43 Adipose tissue is a sizeable and complex endocrine organ that plays a role in metabolic control in
44 part through the secretion of adipokines such as adiponectin and Interleukin-6 (IL-6) (1). Adipose
45 dysfunction is characterised by tissue-specific insulin resistance, local inflammation, fibrosis, and the
46 abnormal secretion of adipokines (2). These adipokines regulate various physiological processes and
47 play a crucial role in the pathophysiology of chronic diseases such as cardiovascular diseases and
48 type 2 diabetes (1). Thus, there is a clear need to develop interventions and strategies that successfully
49 target adipose tissue function.

50

51 Regular prolonged exercise improves various aspects of adipose tissue function, including measures
52 of inflammation and insulin sensitivity (3). This form of prolonged physical activity also has
53 demonstrably important effects on various health outcomes including risk factors for cardiovascular
54 disease and diabetes (4). Unfortunately, many people struggle to undertake such structured exercise
55 and alternative forms of physical activity could potentially play an important role. In many modern
56 societies, people spend much of their time engaged in sedentary behaviours such as using a computer
57 and watching television. In addition to causing a positive energy balance and weight gain, several
58 studies have shown that sedentary behaviour is associated with increased risk of abnormal glucose
59 metabolism, metabolic syndrome, type 2 diabetes and cardiovascular disease (5). Thus, targeting this
60 sedentary behaviour may be important for health.

61

62 In support of this suggestion, short bouts of physical activity lasting just a few minutes spread over
63 the course of a day improve postprandial triglyceridaemia, glycaemia and insulinaemia (6-9).
64 Breaking prolonged sitting with short bouts of physical activity also affects skeletal muscle signalling
65 pathways related to carbohydrate metabolism (8). However, there has been no assessment of whether
66 breaking prolonged sedentary behaviour impacts upon adipose tissue physiology. If this strategy is
67 effective, it would have major implications for the prevention and management of disorders

68 associated with dysfunctional adipose tissue. Thus, this study aims to examine the acute effect of
69 breaking prolonged sedentary behaviour (sitting) on adipose tissue.

70

71 **MATERIALS AND METHODS**

72 **Experimental design**

73 Eleven participants (7 men and 4 post-menopausal women) aged between 35 and 64 years with
74 increased central adiposity were recruited via local advertisement. An initial screening was followed
75 by two subsequent main trials (PROLONGED SITTING and BREAKING SITTING). Trials were
76 conducted in a randomised, counterbalanced design separated by a 3–4 week wash-out period. Two
77 identical meals based on participants' total body mass were provided on the main trial days (morning
78 and lunchtime). Blood was taken regularly during the course of each trial and adipose tissue biopsies
79 were taken at baseline and at the end of each trial. The study protocol was approved by Bristol
80 Research Ethics Committee (REC reference number: 13/SW/0321) and is registered at
81 ClinicalTrials.gov (ID: NCT02870088). All participants provided written informed consent before
82 taking part.

83

84 **Power calculations**

85 There are no data regarding the effect of breaking prolonged sitting on adipose tissue. Adipose tissue
86 is highly responsive to insulin (10) and we have therefore determined sample size based on
87 differences in insulin responses to a standard meal. A previous similar study reported breaking
88 prolonged sitting to reduce postprandial insulinaemia from $3337 \text{ IU} \cdot 9 \text{ h} \cdot \text{L}^{-1}$ to $2470 \text{ IU} \cdot 9 \text{ h} \cdot \text{L}^{-1}$ (9).
89 Based on an estimated SD of $600 \text{ IU} \cdot 9 \text{ h} \cdot \text{L}^{-1}$, we would require 9 participants to show a difference in
90 iAUC for insulin with 95% power and 5% alpha using a 2-tailed paired *t-test*. Eleven participants
91 were recruited to allow for drop out.

92

93

Inclusion and exclusion criteria

Participants were required to be central overweight with at least a waist circumference of 80 cm for women and 94 cm for men (11) and weight stable (no self-reported change in weight $\pm 3\%$) (12) for at least 3 months prior to taking part in this study. Participants completed a health questionnaire to exclude any existing cardiovascular and metabolic diseases and a Physical Activity Readiness Questionnaire (PAR-Q) to ensure that participants were able to walk on the treadmill safely. Participants were asked to walk on a treadmill for 2 min to ensure that this would be tolerated. Smokers, pre-menopausal women and volunteers who used any medications which could influence metabolic and inflammatory responses were excluded. A summary of participants' physical characteristics is shown in Table 1.

[INSERT TABLE 1 ABOUT HERE]

Pre-trial assessments

Physical activity assessment

As part of the pre-trial assessments, participants wore a combined heart rate/accelerometer monitor for 7 days to assess habitual physical activity energy expenditure (Actiheart, Cambridge Neurotechnology Ltd., Cambridge, UK). This was attached to the left of the chest via 2 adhesive ECG pads for 24 h per day except for during showering/bathing/swimming (13).

Body composition analysis

Body mass was assessed using digital scales post-void (TANITA corp., Tokyo, Japan). Waist and hip circumferences were assessed based on World Health Organisation guidelines (11). Body composition was determined by using Dual Energy X-ray Absorptiometry (DEXA; Discovery, Hologic, Bedford, UK). Abdominal subcutaneous and visceral adipose tissue mass was estimated from a central region between L1-L4 (14).

120

121 **Trial days**

122 In the 72 h prior to each main trial, participants were asked to refrain from performing any vigorous-
123 intensity exercise. In the 48 h prior to each main trial, consuming alcohol/caffeine was not allowed
124 and a weighed food and fluid record was completed. Participants were asked to replicate this diet
125 prior to their second main trial. In addition, in the 48 h prior to each main trial, participants were
126 asked to wear a pedometer (Yamax, Japan) to restrict their step counts to under 4,000 steps per day
127 in order to mimic a sedentary lifestyle (15) and eliminate any acute effects from recent physical
128 activity.

129

130 A 12 h fast was performed before arriving at the laboratory on main trial days between 08:00–09:00
131 am. After anthropometric measurements, participants rested on a bed for 15 min, followed by two 5
132 min expired gas sample collections using Douglas bags (Hans Rudolph, MO, USA) to determine
133 resting metabolic rate (RMR) (16) from substrate oxidation as described (17).

134

135 After RMR assessment, a 20-gauge cannula (BD, Venflon™ Pro) was inserted into an antecubital
136 forearm vein and a 10-ml baseline venous blood sample was taken and allocated into tubes with either
137 EDTA or serum separation beads (Sarstedt Ltd, Leicester, UK). Plasma samples were centrifuged
138 immediately at 3,465 g at 4°C for 10 min. Serum samples were left for 45 min to clot before
139 centrifugation. Subcutaneous adipose tissue samples (~1 g) were subsequently taken under local
140 anaesthetic (1 % lidocaine) from the area around the waist approximately 5 cm lateral to the umbilicus
141 with a 14 G needle using an aspiration technique (18) followed by adipose tissue cleaning and
142 processing as previously described (19).

143

144 Two identical meals (breakfast and lunch) were provided and consumed within a 15-min period during
145 each main trial. The first meal (breakfast) was consumed after taking the baseline adipose and blood

sample. The second meal (lunch) was consumed 3 h after the first. The test meal was prescribed according to total body mass and provided 0.35 g fat, 1.17 g carbohydrate, 0.29 g protein and 37 kJ energy per kilogram body mass (6). The percentage of energy from macronutrients was 35 % fat, 52 % carbohydrate and 13 % protein (6). The meal comprised white bread (Hovis; soft white bread, medium sliced), sliced cheese (Sainsbury; cheese slices, basic), butter (Unilever; I can't believe its not butter), mayonnaise (Hellmann; light mayonnaise), lettuce (Sainsbury; Iceberg lettuce), tomato (Sainsbury; tomatoes, basics), ham (Sainsbury; British honey roast), whole milk (Sainsbury; British), cocoa powder (Nesquik; cocoa powder), and yoghurt (Müller; fruit corner strawberry).

154

Prolonged sitting and breaking prolonged sitting

The study protocol is shown in Figure 1. In the PROLONGED SITTING trial, participants sat on a chair for the whole period. In the BREAKING SITTING trial, after consuming the first meal, participants walked 2 min on a treadmill at 6.4 km·h⁻¹ speed (7) every 20 min for the following 180 min. After finishing the second meal, participants continued 2 min of breaking prolonged sitting physical activity at the same speed every 20 min for the following 120 min. For the remainder of the time participants sat on a chair. Thus, in total, participants performed 15 two min bouts of walking throughout the trial (i.e., 30 min of physical activity over 300 min).

163

Ratings of perceived exertion (RPE) and heart rate were collected in the last 30 seconds of each 2-min bout of walking during the breaking sitting trial. Two, 1-min expired air samples were collected during the last minute of walking during the breaking sitting trial to estimate energy expenditure and substrate utilization. In addition, expired air samples were taken using Douglas bags (Hans Rudolph, MO, USA) during two 5-min periods of sitting to calculate total energy expenditure under resting conditions. While sitting, participants were only allowed to read, use a laptop or watch television but were otherwise asked to keep as still as possible throughout (including specific instructions to avoid fidgeting). In the first trial, participants were allowed to consume water *ad libitum* and the volume

172 ingested was replicated for the second trial. In each main trial, baseline blood samples were collected
173 before the first meal and hourly for the following 5 h. Additional blood samples were collected every
174 15 min for the first hour after each meal. A total of 14 blood samples were collected for each trial
175 (Figure 1).

176
177 [INSERT FIGURE 1 ABOUT HERE]

178

179 **Adipose tissue culture and gene expression**

180 After cleaning and mincing the adipose tissue biopsy sample, a 200 mg portion of adipose tissue was
181 homogenised in 5 mL TRIzol (Invitrogen, Paisley, UK) in a 15 mL centrifuge RNase/DNase-free
182 sterile tube (Invitrogen, Paisley, UK) and stored at -80°C for gene expression analysis. The remaining
183 adipose tissue was directly placed in sterile culture plates in duplicate (Nunc, Roskilde, Denmark)
184 with endothelial cell basal media (ECBM) (Promocell, Germany) containing 0.1 % fatty acid-free
185 bovine serum albumin $100\text{ U}\cdot\text{mL}^{-1}$ penicillin and $0.1\text{ mg}\cdot\text{mL}^{-1}$ streptomycin (Sigma-Aldrich,
186 Gillingham, UK). Adipose tissue was incubated with a final ratio of 100 mg tissue per 1 mL ECBM
187 media for 3 h (19). A 37°C , 5 % CO_2 and 95 ± 5 % relative humidity incubator was used (MCO-
188 18A1C CO_2 incubator; Sanyo, Osaka, Japan). After the 3-h incubation, media was transferred to
189 sterile eppendorfs and stored at -80°C . Adipokine secretion from cultured adipose explants was
190 normalised to explant adipose mass and then L1-L4 fat mass as described previously (20).

191

192 **Real-time PCR**

193 An RNeasy Mini Kit (Qiagen, Crawley, UK) was used to extract RNA from TRIzol-digested adipose
194 tissue as described (18). The amount of RNA was quantified using a Qubit 2.0 fluorimeter (Life
195 Technologies, Paisley, UK). RNA was reversed transcribed ($1\text{ }\mu\text{g}$) to cDNA using a High Capacity
196 Reverse Transcription Kit (Applied Biosystems, Warrington, UK). Real-time PCR was performed
197 using a StepOne (Applied Biosystems, Warrington, UK). Predesigned primers and probes were

198 obtained from Applied Biosystems for the measurement of expression of interleukin 6 (IL-6)
 199 (Hs00985639_m1), adiponectin (Hs00605917_m1), leptin (Hs00174877_m1), interleukin 18 (IL-18)
 200 (Hs00155517_m1), tumour necrosis factor alpha (TNF- α) (Hs99999043_m1), monocyte
 201 chemoattractant protein-1 (MCP-1) (Hs00234140_m1), 5' AMP-activated protein kinase (AMPK)
 202 (Hs01562315_m1 and Hs00178903_m1 combined), glucose transporter type 4 (GLUT4)
 203 (Hs00168966_m1), hormone-sensitive lipase (HSL) (Hs00193510_m1), insulin receptor substrate 1
 204 (IRS1) (Hs00178563_m1), insulin receptor substrate 2 (IRS2) (Hs00275843_s1), sterol regulatory
 205 element binding protein 1c (SREBP-1c) (Hs01088691_m1), pyruvate dehydrogenase kinase isozyme
 206 (PDK4) (Hs00176875_m1), peroxisome proliferator-activated receptor γ (PPAR γ)
 207 (Hs01115513_m1), peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 α)
 208 (Hs01016719_m1), RAC-alpha serine/threonine-protein kinase (Akt1) (Hs00178289_m1), adipose
 209 triglyceride lipase (ATGL) (Hs00386101_m1), fatty acid translocase (FAT)/CD36
 210 (Hs00169627_m1), forkhead box protein O1 (FOXO1) (Hs01054576_m1), hexokinase 2 (HK2)
 211 (Hs00606086_m1), phosphoinositide 3-kinase 85 α (PI3K-85 α) (Hs00933163_m1), fatty acid
 212 synthase (FAS) (Hs00188012_m1), peptidylpropyl isomerase A (PPIA) was used as an endogenous
 213 control (21). The comparative Ct method was used to process data where $\Delta Ct = Ct \text{ target gene} - Ct$
 214 PPIA. Ct target genes were normalised to an internal calibrator (lowest ΔCt for each target gene) and
 215 baseline.

216

217 **Biochemical analyses**

218 Concentrations of plasma glucose, plasma triglycerides and serum non-esterified fatty acids were
 219 analysed using commercially available assay kits and analyser (Daytona Rx; Randox, Crumlin, UK).
 220 Serum insulin (Mercodia, Uppsala, Sweden), and both serum and adipose explant secretion of IL-6,
 221 leptin, and adiponectin (R&D systems) were measured using Enzyme-linked immunosorbent assay
 222 (ELISA).

223

224 **Statistical analysis**

225 Descriptive data are presented in text and tables as means \pm standard deviation (SD); variance bars
226 on figures are presented as means and standard error of the mean (SEM). Time series data were
227 examined using a two-way ANOVA (trial \times time) with repeated measures using SPSS version 22
228 (IBM, Armonk, NY, USA). Green-house–Geisser corrections were applied to intra-individual
229 contrasts where $\varepsilon < 0.75$; however, for less severe asphericity the Huynh–Feldt correction was
230 selected (22) . Incremental area under curve (iAUC) was calculated using the trapezoid method (23)
231 and the differences in summative scores between trials were analysed using paired *t-tests*. Data for
232 iAUC represent the period from the consumption of the first meal to the conclusion of the second
233 meal (330 min). Analysis of iAUCs and gene expression data were conducted using logged
234 transformed data as previously described (18). Statistical significance was set at $p \leq 0.05$.

235 RESULTS

236 Blood glucose, insulin, triglyceride and NEFA concentrations in prolonged and breaking sitting 237 trials

238 Trial \times Time interaction effects were found for blood glucose and insulin between PROLONGED
239 and BREAKING SITTING trials (all $p \leq 0.05$, Figure 2A and C). The glucose and insulin iAUC were
240 attenuated significantly by BREAKING SITTING compared to PROLONGED SITTING (359 ± 117
241 *versus* 697 ± 218 mmol \cdot 330 min \cdot L $^{-1}$, $p = 0.001$ and 202 ± 71 *versus* 346 ± 150 nmol \cdot 330 min \cdot L $^{-1}$, p
242 $= 0.001$, respectively, Figure 2B and D). There was no interaction effect for blood triglyceride and
243 NEFA (Figure 2E and G). Triglyceride iAUC was significantly higher in BREAKING SITTING
244 compared to PROLONGED SITTING trial (283 ± 36 vs. 232 ± 32 mmol \cdot 330 min \cdot L $^{-1}$, $p = 0.002$,
245 respectively, Figure 2F).

246
247 [INSERT FIGURE 2 ABOUT HERE]

248 249 Adipose tissue mRNA gene expression

250 There was no interaction effect (trial \times time) in adipose tissue mRNA gene expression between
251 BREAKING SITTING and PROLONGED SITTING trials for any of the genes analysed (Figure 3).
252 However, there were time effects for IL-6, MCP1, PDK4, IRS1 and 2, PI3K, HSL and SREBP1c (all
253 $p \leq 0.05$, Figure 3).

254
255 [INSERT FIGURE 3 ABOUT HERE]

256
257

258 **Serum adipokine concentrations and adipose tissue adipokine secretion *ex vivo***

259 There was no difference between PROLONGED SITTING and BREAKING SITTING in serum IL-
260 6, leptin and adiponectin (Figure 4A, C and E) or the secretion *ex vivo* of IL-6, leptin and adiponectin
261 (Figure 4B, D and F). There was a time effect for serum IL-6 and leptin ($p \leq 0.05$) (Figure 4).

262
263 [INSERT FIGURE 4 ABOUT HERE]

264
265 **Pre-trial physical activity and resting metabolic rate measures**

266 The average of 48 h step counts prior to PROLONGED and BREAKING SITTING trials was not
267 different ($3,868 \pm 1,304$ *versus* $3,669 \pm 1,090$ steps·day⁻¹, respectively) and there was no difference
268 in pre-fed RMR on the trial day between trials ($1,882 \pm 303$ *versus* $1,833 \pm 338$ kcal·day⁻¹,
269 respectively).

270
271 **Physiological response during trials**

272 During the 15 bouts of two min walking, the average heart rate was 135 ± 12 beats· min⁻¹ with an
273 RPE (6–20 scale) of 10 ± 2 . The difference in energy expenditure between trials was 216 kcal over
274 the 5.5 h period (Table 2).

275
276 [INSERT TABLE 2 ABOUT HERE]

277

278 **DISCUSSION**

279 The present study investigated whether breaking sitting with regular short bouts of walking influences
280 adipose tissue gene expression and secretion of selected proteins from adipose tissue *ex vivo*. We
281 found that regularly participating in short bouts of walking reduced postprandial glycaemic and
282 insulinaemic responses by 48% and 42%, respectively. However, despite the profound difference in
283 postprandial insulin and glucose concentrations, changes in subcutaneous adipose gene expression
284 were similar in both trials. There was also no difference in adipose tissue explant secretion and
285 circulating concentrations of IL-6, adiponectin or leptin.

286

287 **The effect of breaking sitting on postprandial glycaemia and insulinaemia**

288 The extent of postprandial glucose and insulin excursions are associated with cardiovascular disease
289 (24, 25). Therefore, strategies to attenuate postprandial glucose and insulin responses would be
290 extremely useful. Breaking prolonged sitting has been shown to reduce postprandial glucose and
291 insulin levels in most (7, 9, 26) but not all studies (27). In the present study, we found that
292 participating in regular short bouts of walking attenuated postprandial glucose and insulin
293 concentrations in sedentary middle-aged central overweight men and women (glucose and insulin
294 iAUC's were attenuated by 48% and 42%, respectively). The additional energy cost of walking during
295 the BREAKING SITTING trial was supported by carbohydrate oxidation and thus we propose that
296 oxidation of carbohydrate may be partly responsible for the relatively lower blood glucose observed
297 in this trial. Thus, breaking prolonged sitting is very effective at reducing postprandial glycaemia and
298 insulinaemia.

299

300 **Adipose tissue responses to breaking prolonged sitting**

301 In skeletal muscle, breaking sitting with either light or moderate-intensity walking increases the
302 expression of genes involved in glucose metabolism, including PDK4 (8). In the present study, we
303 did not find any differences between PROLONGED SITTING and BREAKING SITTING trials in

304 the expression of these genes in adipose even though our walking speed, frequency and duration was
305 very similar to that previous study (8). Thus, it appears that acutely breaking prolonged sitting with
306 short bouts of walking reduces postprandial glycaemia/insulinaemia and leads to corresponding
307 changes in key pathways in skeletal muscle; whereas a similar effect on postprandial
308 glycaemia/insulinaemia is not associated with corresponding changes within adipose tissue. These
309 divergent effects are likely to reflect the different roles of these tissues. Skeletal muscle plays a major
310 role in the disposal of exogenously ingested glucose (especially during physical activity), whereas
311 adipose tissue plays a relatively minor role in the acute regulation of blood glucose concentrations
312 (28). Instead, during the postprandial period, adipose tissue plays a major role in responding to
313 feeding and storing ingested lipids (29). Given the effect of glucose and insulin infusion on adipose
314 tissue gene expression (10, 30), it is possible that the magnitude of postprandial glycaemia and
315 insulinaemia in the present study was a more-than-adequate stimulus for adipose tissue, even during
316 the BREAKING SITTING trial. We have recently shown that feeding exerts a potent effect on
317 adipose tissue and can blunt the normal responses to prolonged structured exercise (20). Thus, we
318 propose that the effect of feeding is likely to overcome the impact of physical activity on adipose
319 tissue – whether this is during repeated short bouts of physical activity to break prolonged sitting or
320 during more prolonged structured exercise.

321

322 There was an apparent increase in triglyceride iAUC with BREAKING SITTING trial and this may
323 (ostensibly) appear to be a negative response. However, this is likely explained by an enhanced lipid
324 mobilisation in the BREAKING SITTING trial combined with technical considerations related to the
325 assay used for the assessment of plasma triglyceride. Our method for the assessment of plasma
326 triglyceride does not discriminate between free glycerol, bound glycerol in chylomicrons and very-
327 low-density lipoprotein; and thus it is likely that the greater triglyceride iAUC in the BREAKING
328 SITTING trial reflects an increase in circulating free glycerol due to adipose tissue lipolysis.

329

330 **Temporal changes in adipose tissue gene expression after feeding**

331 Even though we did not observe any differences in gene expression between BREAKING and
332 PROLONGED SITTING trials, there were temporal changes in both trials that could reflect the effect
333 of feeding *per se*. The expression of adipose SREBP1c was increased in both trials and this response
334 is probably indicative of lipid synthesis after feeding (31). Moreover, there was a decrease in HSL
335 and PDK4 in both trials, which may reflect a reduction in lipid mobilisation and oxidation. In addition,
336 insulin receptor, IRS1 and IRS2 gene expression were consistently up-regulated and down-regulated
337 respectively at the end of both trials. IRS-1 plays a central role as an insulin signalling receptor (32)
338 and, similar to the present study, adipose IRS-2 mRNA expression was down-regulated in lean,
339 overweight and obese populations after feeding (33). Taken together, the changes in SREBP1c, HSL,
340 PDK4 and IRS-1 and IRS-2 in both trials probably reflects the normal physiological and metabolic
341 response to feeding.

342

343 We also observed an increase in adipose IL-6 and MCP-1 in both trials. Other studies have shown
344 that adipose IL-6 and MCP-1 gene expression were up-regulated after meal consumption in lean,
345 overweight and obese men (33), in people with metabolic syndrome (34), in people with type 2
346 diabetes (35) and in healthy lean populations (36). A meal-induced up-regulation of adipose IL-6 and
347 MCP-1 gene expression might support the notion that feeding induces acute transient adipose tissue
348 inflammation. However, we cannot discount the possibility that these changes could also reflect an
349 adipose circadian rhythm (37).

350

351 **Experimental Design Considerations**

352 In the present study, only two adipose biopsies were taken and therefore we do not have a complete
353 time-course of whether the temporal changes observed in both trials was due to meal consumption
354 and/or an entrained biological rhythm. Repeated adipose biopsies might help resolve this issue but
355 this could be problematic due to potential confounding from local inflammation (36). We should also

356 emphasise that each condition (trial arm) was conducted over the course of a single day. Given the
357 large reduction in serum insulin concentrations in the BREAKING SITTING trial and the powerful
358 effect of insulin on adipose tissue (10, 38), it is possible that if this intervention was repeated for
359 multiple days and/or months then this difference in postprandial insulin would lead to chronic changes
360 in adipose in the longer term. We also do not know how the present results would compare to the
361 same duration of exercise but performed in a different way (e.g., a single bout of 30 min or three
362 bouts of 10 min). In the present study, we provided two mixed meals with a total ~1,725 kcal, which
363 is more than 2-fold the energy intake in the single meal provided by Latouche et al (8). Given the
364 extended timescale ~1,725 kcal is not an unreasonable amount of energy to consume (39), but it is
365 possible that the physiological pressure of processing and storing this amount of energy might
366 outweigh the impact of small bouts of physical activity in adipose tissue. Had we provided smaller
367 meals, it is possible that physical activity could have had a moderating effect on adipose tissue-related
368 outcomes.

369

370 **Conclusions**

371 Whilst breaking prolonged sitting is an effective strategy for acutely managing postprandial
372 glycaemia and insulinaemia, this does not elicit corresponding temporal changes in adipose tissue.

373 **Acknowledgements**

374 This project was funded by Ministry of Education (Taiwan) and the University of Bath. We thank all
375 the participants for their time and effort to take part in this project.

376

377 **Conflict of Interest**

378 The authors declare no competing interests. The results of the present study do not constitute
379 endorsement by the American College of Sports Medicine. The results of this study are presented
380 clearly, honestly, and without fabrications, falsification, or inappropriate data manipulation.

381

382 **Author contributions**

383 Yung-Chih Chen was responsible for study design and conduct, data collection, data analysis, data
384 interpretation, statistical analysis and manuscript revision; James Betts was responsible for study
385 design and manuscript revision; Jean-Philippe Walhin assisted with adipose tissue biopsies and
386 manuscript revision. Dylan Thompson was responsible for funding, study design, data interpretation
387 and manuscript revision.

388 **References Uncategorized References**

- 389 1. Piya MK, McTernan PG, Kumar S. Adipokine inflammation and insulin resistance: the role of
390 glucose, lipids and endotoxin. *The Journal of endocrinology*. 2013;216(1):T1-T15. doi: 10.1530/JOE-12-
391 0498. PubMed PMID: 23160966.
- 392 2. Langin D, Frühbeck G, Frayn KN, Lafontan M. Adipose tissue: development, anatomy and functions.
393 In: Williams G, Frühbeck G, editors. *Obesity: Science to Practice*. Chichester, UK: Wiley-Blackwell; 2009. p.
394 79-108.
- 395 3. Thompson D, Karpe F, Lafontan M, Frayn K. Physical activity and exercise in the regulation of
396 human adipose tissue physiology. *Physiological reviews*. 2012;92(1):157-91. doi:
397 10.1152/physrev.00012.2011. PubMed PMID: 22298655.
- 398 4. Sakurai T, Ogasawara J, Kizaki T, Sato S, Ishibashi Y, Takahashi M, et al. The effects of exercise
399 training on obesity-induced dysregulated expression of adipokines in white adipose tissue. *International*
400 *journal of endocrinology*. 2013;2013:801743. doi: 10.1155/2013/801743. PubMed PMID: 24369466;
401 PubMed Central PMCID: PMC3867917.
- 402 5. Wilmot EG, Edwardson CL, Achana FA, Davies MJ, Gorely T, Gray LJ, et al. Sedentary time in adults
403 and the association with diabetes, cardiovascular disease and death: systematic review and meta-analysis.
404 *Diabetologia*. 2012;55(11):2895-905. doi: 10.1007/s00125-012-2677-z. PubMed PMID: 22890825.
- 405 6. Miyashita M. Effects of continuous versus accumulated activity patterns on postprandial
406 triacylglycerol concentrations in obese men. *International journal of obesity*. 2008;32(8):1271-8. doi:
407 10.1038/ijo.2008.73. PubMed PMID: 18504443.
- 408 7. Dunstan DW, Kingwell BA, Larsen R, Healy GN, Cerin E, Hamilton MT, et al. Breaking up prolonged
409 sitting reduces postprandial glucose and insulin responses. *Diabetes care*. 2012;35(5):976-83. doi:
410 10.2337/dc11-1931. PubMed PMID: 22374636; PubMed Central PMCID: PMC3329818.
- 411 8. Latouche C, Jowett JB, Carey AL, Bertovic DA, Owen N, Dunstan DW, et al. Effects of breaking up
412 prolonged sitting on skeletal muscle gene expression. *J Appl Physiol (1985)*. 2013;114(4):453-60. doi:
413 10.1152/jappphysiol.00978.2012. PubMed PMID: 23271697.
- 414 9. Peddie MC, Bone JL, Rehrer NJ, Skeaff CM, Gray AR, Perry TL. Breaking prolonged sitting reduces
415 postprandial glycemia in healthy, normal-weight adults: a randomized crossover trial. *The American journal*
416 *of clinical nutrition*. 2013;98(2):358-66. doi: 10.3945/ajcn.112.051763. PubMed PMID: 23803893.
- 417 10. Krogh-Madsen R, Plomgaard P, Keller P, Keller C, Pedersen BK. Insulin stimulates interleukin-6 and
418 tumor necrosis factor-alpha gene expression in human subcutaneous adipose tissue. *American journal of*
419 *physiology Endocrinology and metabolism*. 2004;286(2):E234-8. doi: 10.1152/ajpendo.00274.2003.
420 PubMed PMID: 14532168.
- 421 11. WHO. World Health Organisation-Waist Circumference and Waist-Hip Ratio: Report of a WHO
422 Expert Consultation. 2008.
- 423 12. Stevens J, Truesdale KP, McClain JE, Cai J. The definition of weight maintenance. *International*
424 *journal of obesity*. 2006;30(3):391-9. doi: 10.1038/sj.ijo.0803175. PubMed PMID: 16302013.
- 425 13. Thompson D, Batterham AM, Bock S, Robson C, Stokes K. Assessment of low-to-moderate intensity
426 physical activity thermogenesis in young adults using synchronized heart rate and accelerometry with
427 branched-equation modeling. *The Journal of nutrition*. 2006;136(4):1037-42. PubMed PMID: 16549471.
- 428 14. Glickman SG, Marn CS, Supiano MA, Dengel DR. Validity and reliability of dual-energy X-ray
429 absorptiometry for the assessment of abdominal adiposity. *Journal of applied physiology*. 2004;97(2):509-
430 14. doi: 10.1152/jappphysiol.01234.2003. PubMed PMID: 15075304.
- 431 15. Tudor-Locke C, Craig CL, Thyfault JP, Spence JC. A step-defined sedentary lifestyle index: <5000
432 steps/day. *Applied physiology, nutrition, and metabolism = Physiologie appliquee, nutrition et*
433 *metabolisme*. 2013;38(2):100-14. doi: 10.1139/apnm-2012-0235. PubMed PMID: 23438219.
- 434 16. Compher C, Frankenfield D, Keim N, Roth-Yousey L, Evidence Analysis Working G. Best practice
435 methods to apply to measurement of resting metabolic rate in adults: a systematic review. *Journal of the*
436 *American Dietetic Association*. 2006;106(6):881-903. doi: 10.1016/j.jada.2006.02.009. PubMed PMID:
437 16720129.
- 438 17. Frayn KN. Calculation of substrate oxidation rates in vivo from gaseous exchange. *Journal of applied*
439 *physiology: respiratory, environmental and exercise physiology*. 1983;55(2):628-34. PubMed PMID:
440 6618956.

- 441 18. Walhin JP, Richardson JD, Betts JA, Thompson D. Exercise counteracts the effects of short-term
442 overfeeding and reduced physical activity independent of energy imbalance in healthy young men. *The*
443 *Journal of physiology*. 2013;591(24):6231-43. doi: 10.1113/jphysiol.2013.262709. PubMed PMID:
444 24167223; PubMed Central PMCID: PMC3892474.
- 445 19. Travers RL, Motta AC, Betts JA, Bouloumie A, Thompson D. The impact of adiposity on adipose
446 tissue-resident lymphocyte activation in humans. *International journal of obesity*. 2015;39(5):762-9. doi:
447 10.1038/ijo.2014.195. PubMed PMID: 25388403; PubMed Central PMCID: PMC4424387.
- 448 20. Chen YC, Travers RL, Walhin JP, Gonzalez JT, Koumanov F, Betts JA, et al. Feeding influences adipose
449 tissue responses to exercise in overweight men. *American journal of physiology Endocrinology and*
450 *metabolism*. 2017;313(1):E84-E93. doi: 10.1152/ajpendo.00006.2017. PubMed PMID: 28292758.
- 451 21. Neville MJ, Collins JM, Gloyn AL, McCarthy MI, Karpe F. Comprehensive human adipose tissue
452 mRNA and microRNA endogenous control selection for quantitative real-time-PCR normalization. *Obesity*.
453 2011;19(4):888-92. doi: 10.1038/oby.2010.257. PubMed PMID: 20948521; PubMed Central PMCID:
454 PMC4623139.
- 455 22. Atkinson G. Analysis of repeated measurements in physical therapy research. *Phys Ther Sport*.
456 2001;2(4):194-208.
- 457 23. Wolever TM, Jenkins DJ. The use of the glycemic index in predicting the blood glucose response to
458 mixed meals. *The American journal of clinical nutrition*. 1986;43(1):167-72. PubMed PMID: 3942088.
- 459 24. Cavalot F, Petrelli A, Traversa M, Bonomo K, Fiora E, Conti M, et al. Postprandial blood glucose is a
460 stronger predictor of cardiovascular events than fasting blood glucose in type 2 diabetes mellitus,
461 particularly in women: lessons from the San Luigi Gonzaga Diabetes Study. *The Journal of clinical*
462 *endocrinology and metabolism*. 2006;91(3):813-9. doi: 10.1210/jc.2005-1005. PubMed PMID: 16352690.
- 463 25. Bonora E, Muggeo M. Postprandial blood glucose as a risk factor for cardiovascular disease in Type
464 II diabetes: the epidemiological evidence. *Diabetologia*. 2001;44(12):2107-14. doi:
465 10.1007/s001250100020. PubMed PMID: 11793012.
- 466 26. Henson J, Davies MJ, Bodicoat DH, Edwardson CL, Gill JM, Stensel DJ, et al. Breaking Up Prolonged
467 Sitting With Standing or Walking Attenuates the Postprandial Metabolic Response in Postmenopausal
468 Women: A Randomized Acute Study. *Diabetes care*. 2016;39(1):130-8. doi: 10.2337/dc15-1240. PubMed
469 PMID: 26628415.
- 470 27. Saunders TJ, Chaput JP, Goldfield GS, Colley RC, Kenny GP, Doucet E, et al. Prolonged sitting and
471 markers of cardiometabolic disease risk in children and youth: a randomized crossover study. *Metabolism:*
472 *clinical and experimental*. 2013;62(10):1423-8. doi: 10.1016/j.metabol.2013.05.010. PubMed PMID:
473 23773981.
- 474 28. Im SS, Kwon SK, Kim TH, Kim HI, Ahn YH. Regulation of glucose transporter type 4 isoform gene
475 expression in muscle and adipocytes. *IUBMB life*. 2007;59(3):134-45. doi: 10.1080/15216540701313788.
476 PubMed PMID: 17487684.
- 477 29. Ruge T, Hodson L, Cheeseman J, Dennis AL, Fielding BA, Humphreys SM, et al. Fasted to fed
478 trafficking of Fatty acids in human adipose tissue reveals a novel regulatory step for enhanced fat storage.
479 *The Journal of clinical endocrinology and metabolism*. 2009;94(5):1781-8. doi: 10.1210/jc.2008-2090.
480 PubMed PMID: 19223522.
- 481 30. Meugnier E, Faraj M, Rome S, Beauregard G, Michaut A, Pelloux V, et al. Acute hyperglycemia
482 induces a global downregulation of gene expression in adipose tissue and skeletal muscle of healthy
483 subjects. *Diabetes*. 2007;56(4):992-9. doi: 10.2337/db06-1242. PubMed PMID: 17303805.
- 484 31. Shimano H. Sterol regulatory element-binding protein family as global regulators of lipid synthetic
485 genes in energy metabolism. *Vitamins and hormones*. 2002;65:167-94. PubMed PMID: 12481547.
- 486 32. Thirone AC, Huang C, Klip A. Tissue-specific roles of IRS proteins in insulin signaling and glucose
487 transport. *Trends in endocrinology and metabolism: TEM*. 2006;17(2):72-8. doi:
488 10.1016/j.tem.2006.01.005. PubMed PMID: 16458527.
- 489 33. Travers RL, Motta AC, Betts JA, Thompson D. Adipose tissue metabolic and inflammatory responses
490 to a mixed meal in lean, overweight and obese men. *European journal of nutrition*. 2015. doi:
491 10.1007/s00394-015-1087-7. PubMed PMID: 26514561.
- 492 34. Meneses ME, Camargo A, Perez-Martinez P, Delgado-Lista J, Cruz-Teno C, Jimenez-Gomez Y, et al.
493 Postprandial inflammatory response in adipose tissue of patients with metabolic syndrome after the intake

494 of different dietary models. *Molecular nutrition & food research*. 2011;55(12):1759-70. doi:
 495 10.1002/mnfr.201100200. PubMed PMID: 22144044.

496 35. Pietraszek A, Gregersen S, Hermansen K. Acute effects of dietary fat on inflammatory markers and
 497 gene expression in first-degree relatives of type 2 diabetes patients. *The review of diabetic studies : RDS*.
 498 2011;8(4):477-89. doi: 10.1900/RDS.2011.8.477. PubMed PMID: 22580729; PubMed Central PMCID:
 499 PMC3359692.

500 36. Dordevic AL, Pendergast FJ, Morgan H, Villas-Boas S, Caldow MK, Larsen AE, et al. Postprandial
 501 Responses to Lipid and Carbohydrate Ingestion in Repeated Subcutaneous Adipose Tissue Biopsies in
 502 Healthy Adults. *Nutrients*. 2015;7(7):5347-61. doi: 10.3390/nu7075224. PubMed PMID: 26140541; PubMed
 503 Central PMCID: PMC4517001.

504 37. Johnston JD. Adipose circadian rhythms: translating cellular and animal studies to human
 505 physiology. *Molecular and cellular endocrinology*. 2012;349(1):45-50. doi: 10.1016/j.mce.2011.05.008.
 506 PubMed PMID: 21664232.

507 38. Laviola L, Perrini S, Cignarelli A, Natalicchio A, Leonardini A, De Stefano F, et al. Insulin signaling in
 508 human visceral and subcutaneous adipose tissue in vivo. *Diabetes*. 2006;55(4):952-61. PubMed PMID:
 509 16567516.

510 39. Chowdhury EA, Richardson JD, Tsintzas K, Thompson D, Betts JA. Carbohydrate-rich breakfast
 511 attenuates glycaemic, insulinaemic and ghrelin response to ad libitum lunch relative to morning fasting in
 512 lean adults. *The British journal of nutrition*. 2015;114(1):98-107. doi: 10.1017/S0007114515001506.
 513 PubMed PMID: 26004166; PubMed Central PMCID: PMC4530602.

514

515 **Figure Legends**

516

517 **Figure 1. Experimental protocol in PROLONGED SITTING and BREAKING SITTING trials.**

518 In the PROLONGED SITTING trial, participants sat on a chair throughout. In the BREAKING
519 SITTING trial, participants walked on a treadmill at $6.4 \text{ km} \cdot \text{h}^{-1}$ for 2 min every 20 min.

520

521 **Figure 2: Blood glucose (A), insulin (C), triglyceride (E) and NEFA (G) concentrations in**
522 **PROLONGED SITTING and BREAKING SITTING trials. iAUC for glucose (B), insulin (D)**

523 **and triglyceride (F).** iAUC is not shown for NEFA because values in both trials are mostly below
524 baseline. The sample size is $n = 10$ due to difficulty in cannulating one female participant. Values are
525 means \pm SEM. # denotes significant interaction effect between PROLONGED SITTING and
526 BREAKING SITTING trials. * denotes significantly different between prolonged sitting *versus*
527 breaking sitting trials ($p \leq 0.05$). The shaded box denotes meal time. Dashed lines with grey colour
528 denotes female.

529

530 **Figure 3. Fold changes in relative adipose tissue gene expression under PROLONGED**
531 **SITTING and BREAKING SITTING trials** (all $n = 9$, due to lack of sufficient tissue samples for

532 two participants). The dashed line indicates baseline. Data normalised to PPIA, internal calibrator
533 and baseline. Samples below the detectable limit ($\text{Ct} > 35$) were excluded from the analysis. † denotes
534 significant time effect and * denotes significant trial effect ($p \leq 0.05$). Values are means \pm SEM.

535

536 **Figure 4: Circulating serum IL-6 (A) leptin (C) and adiponectin (D) concentrations in**
537 **PROLONGED SITTING and BREAKING SITTING trials** (sample size is $n = 10$ due to difficulty

538 in cannulating one participant). **Adipose explant protein secretion of IL-6 (B), leptin (D) and**
539 **adiponectin (F) at baseline (AM) and at end of the trial (PM)** (all $n = 9$, due to lack of sufficient

540 tissue samples for one male and one female participant). † denotes a significant time effect ($p \leq$

541 0.05). Values are means \pm SEM. The shaded box denotes meal time. Dashed lines with grey colour
542 denotes female.
543

544 **Table 1. Participants physical characteristics (n = 11)**

Characteristics	Mean ± SD
Age (years)	50 ± 5
Body mass (kg)	97.6 ± 20.8
Height (m)	1.73 ± 0.08
Waist circumference (cm)	109 ± 14
Hip circumference (cm)	112 ± 12
Body mass index (kg·m ⁻²)	32.5 ± 6.7
Fat mass (%)	35 ± 6
Fat mass (kg)	33.8 ± 11.0
Fat in L1-L4 region (kg; DEXA)	4.7 ± 1.9
Physical activity level (PAL)	1.48 ± 0.16
Systolic blood pressure (mmHg)	137 ± 12
Diastolic blood pressure (mmHg)	89 ± 7

545 Fat mass in L1-L4 region was assessed as described previously (14).

546

547 **Table 2.** Energy expenditure and substrate oxidation during each 330 min trial (n = 11)

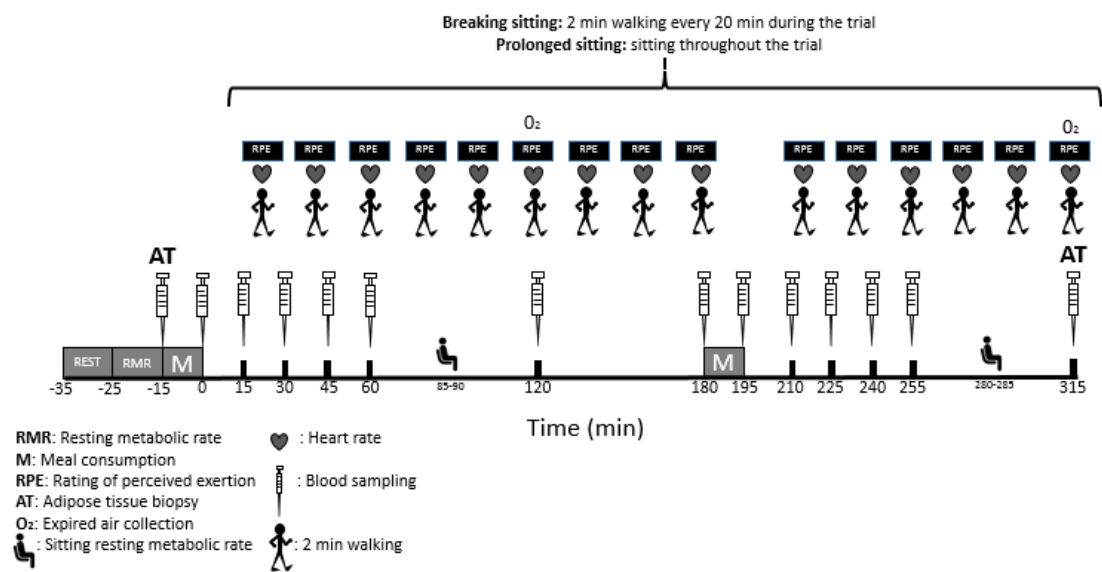
	PROLONGED	BREAKING
	SITTING	SITTING
Energy expenditure (kcal) *	577 ± 129	793 ± 149
Carbohydrate oxidation (g) *	112 ± 45	168 ± 48
Lipid oxidation (g)	17 ± 13	18 ± 10

548 Values are means ± SD. * denotes significantly different between PROLONGED SITTING *versus*
549 BREAKING SITTING trials ($p \leq 0.05$).

550

551

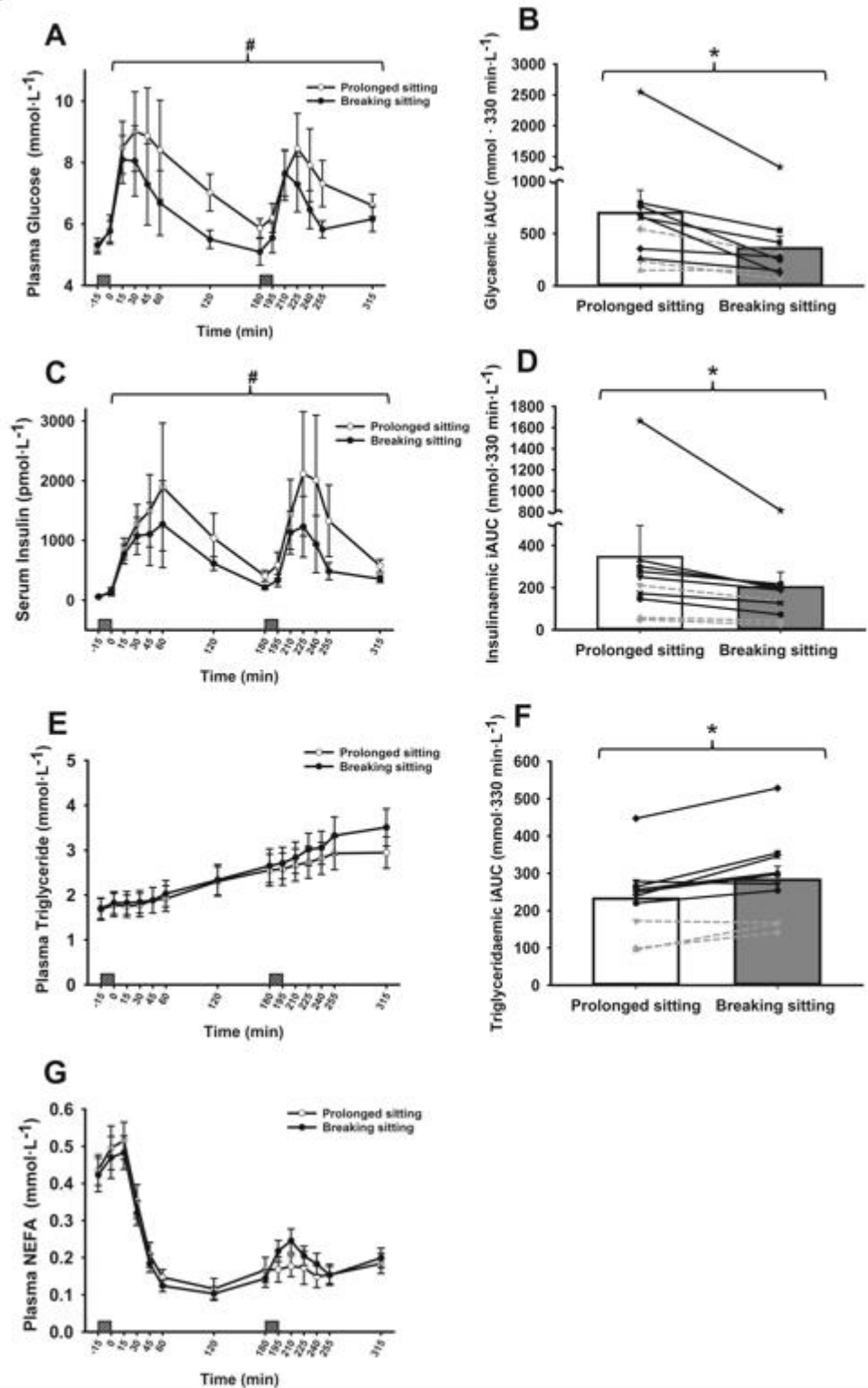
Figure 1



552

553

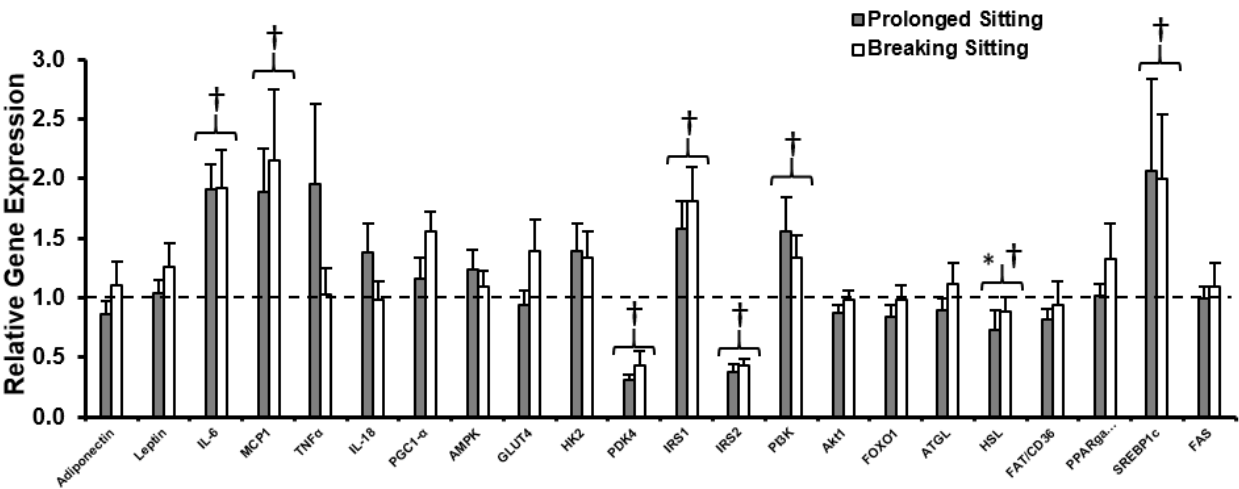
Figure 2



554

555

Figure 3



556

557

Figure 4

